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PROTEIN ISOLATE FROM HIGH-PROTEIN OATS: PREPARATION, COMPOSITION AND PROPERTIES

ABSTRACT

An alkaline extraction process was developed to produce protein isolates and starch from defatted flour or defatted groats of high-protein oats. Optimum extraction was at pH 9.2 in 0.02N sodium hydroxide with a solid:solvent ratio of 1:6. The defatted flour was extracted with sodium hydroxide solution, and bran was removed by screening the alkaline dispersion. After centrifuging the slurry that passed through the screen, the alkaline supernatant was adjusted to pH 5.0 to yield a precipitate (protein isolate) and supernatant. The defatted groats were first extracted with water followed by two sodium hydroxide extractions. Bran was removed by screening the second alkaline dispersion, and protein isolate was precipitated from the first alkaline extract at pH 5.7. Protein content (nitrogen × 6.25) of the isolate varied between 94 and 103% and accounted for 53–67% of total protein from defatted flour or groats. The isolate contained from 3.4–4.0g lysine and 2.2–4.2g total sulfur amino acids per 16g nitrogen. Minimum nitrogen solubility of the isolates was 3–4% near pH 5.5, and solubility was 78–83% near pH 2.2. All protein isolates had good hydration capacity (2.9–3.9) and two of the isolates had good emulsifying activity (around 50%) and good emulsion stability (near 50%).

INTRODUCTION

OATS HAVE good quality and relatively high protein content compared with other cereal grains (Jones et al., 1948; Hischke et al., 1968; Robbins et al., 1971). In contrast to most cereal grains, oats maintain a good amino acid balance as protein content is increased by genetic selection (Robbins et al., 1971), and this was demonstrated by amino acid analyses and chick and rat growth experiments (Maruyama et al., 1975).

Concentrates with 59–75% protein were prepared from ground groats by a wet milling process (Cluskey et al., 1973; Wu et al., 1973). Fractions with 50–57% protein were obtained by extraction of groat bran and flour (Youngs, 1974). We investigated a number of factors affecting extraction of isolate (more than 90% protein) from defatted oat groats and flour of high protein content. We determined protein, starch, fiber, fat, ash, and amino acid composition of oat isolate and by-products, as well as nitrogen solubility, hydration capacity, emulsifying activity, and emulsion stability of the isolate.

EXPERIMENTAL

Protein and nitrogen determinations

Nitrogen was determined by duplicate micro-Kjeldahl, and protein was calculated from nitrogen × 6.25. All protein and nitrogen values were on a dry basis.

Oats

Garland oats (lot BH-474) (grown in Minnesota in 1970) were purchased from Interstate Seed and Grain Co., Fargo, ND. They had an unusually high protein content (17.2%) for this particular variety. Dal oats, a gift from H.L. Shands, Dept. of Agronomy, University of Wisconsin, Madison, were grown in Wisconsin in 1971 and also had high protein content (17.8%).

An Alpine pin mill at 1445 rpm was used to dehull the oats, and the resulting groats were separated from hulls by screening and aspiration. The groats were then ground in a hammer mill. Some of the Garland groats were defatted with 1-butanol (B), followed by a hexane wash to remove any remaining 1-butanol, whereas some were defatted only with hexane (H). Dal groats were defatted only with hexane.

The defatted Garland groats were separated by a 100-mesh screen, and the fraction that passed through the screen (flour) represented 61% of the hexane-defatted groats and 56% of the 1-butanol-defatted groats.

Protein extraction and precipitation

Defatted Garland flour was mixed with various solvents at a solid: solvent ratio of 1:6, stirred for 25 min and centrifuged for 15 min at 3300 × G. The supernatant was then freeze dried.

An alkaline extract (5 ml) of defatted Garland flour was pipetted into each of five centrifuge tubes, and hydrochloric acid was added dropwise to each tube until pH values were 5.0, 5.5, 6.0, 6.5 and 7.0. The mixture in each tube was stirred magnetically for 25 min and centrifuged at 3300 × G for 10 min. A portion of each supernatant after centrifugation was analyzed for nitrogen. The maximum amount of protein was precipitated at pH 5.0.

Protein isolate

Defatted Garland flour (60g) and 360 ml of 0.02N sodium hydroxide were stirred for 25 min (Fig. 1). The slurry at pH 9.2 was then passed through 100-mesh bolting cloth to remove bran. The slurry that passed through the cloth was centrifuged at 3300 × G for 15 min to obtain a supernatant, a starch layer and a layer above starch. The supernatant was adjusted to pH 5.0 by addition of hydrochloric acid solution to precipitate almost all the protein. The mixture was centrifuged at 3300 × G for 15 min to yield a precipitate and a supernatant, which were freeze-dried separately to get protein isolate and supernatant. The starch, the layer above the starch and the bran that remained on the bolting cloth were each neutralized with hydrochloric acid solution and freeze dried.

Hexane-defatted Dal groats (12 kg) and 72 liters of water were mixed with an air-driven stirrer for 25 min. The mixture was centrifuged (Fig. 2) in a Sharples super centrifuge at 12,800 × G to yield a supernatant and a solid. The supernatant was freeze dried and named water extract.

The original volume of groats plus water was restored by addition of water and sodium hydroxide solution to the solid (Fig. 2). The alkaline slurry at pH 9.2 was stirred for 25 min and centrifuged to yield a supernatant and a solid. The supernatant was adjusted to pH 5.7 to precipitate almost all the protein. The mixture was then centrifuged to

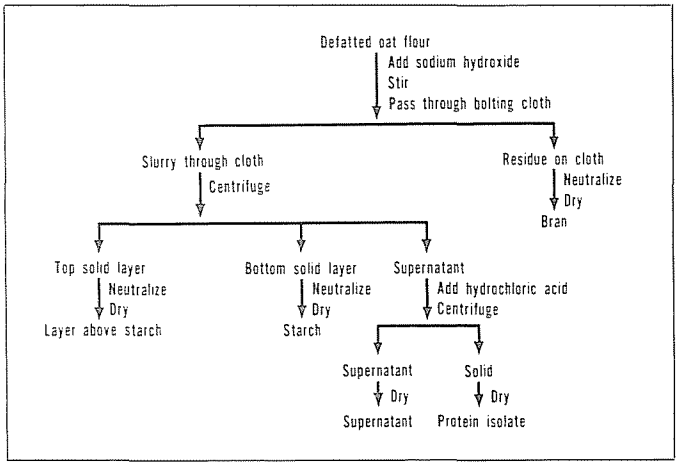

Fig. 1—Schematic diagram for preparing protein isolate and by-products from defatted oat flour by alkaline extraction.

yield a solid and a supernatant, which were freeze dried separately to give a protein isolate (first precipitate) and a first supernatant.

The original volume and pH were again restored by addition of water and sodium hydroxide solution to the alkaline solid (Fig. 2). This alkaline slurry was stirred for 25 min and passed through 100-mesh bolting cloth to remove bran. The slurry that passed through the cloth was centrifuged to yield a supernatant and a solid starch layer. The supernatant was adjusted to pH 5.7 by addition of hydrochloric acid solution to precipitate almost all the protein. The mixture was then centrifuged to yield a supernatant and a solid, and subsequently freeze dried separately to give a second supernatant and a second precipitate. The bran that remained on the bolting cloth and the starch were each neutralized with hydrochloric acid solution and dried.

Table 1—Influence of solvent and pH on extraction of protein from defatted Garland flour (solid:solvent ratio 1:6)

Solvent	pH of slurry	Nitrogen extracted (%)	Protein in extract solids (% dry basis)
0.04N HCl	2.5	39	65
Water	6.2	9	41
0.005N NaOH	7.4	14	33
0.01N NaOH	8.6	27	60
0.02N NaOH	9.1	67	90
0.03N NaOH	9.8	71	88
0.04N NaOH	10.8	75	78
0.05N NaOH	11.1	69	85

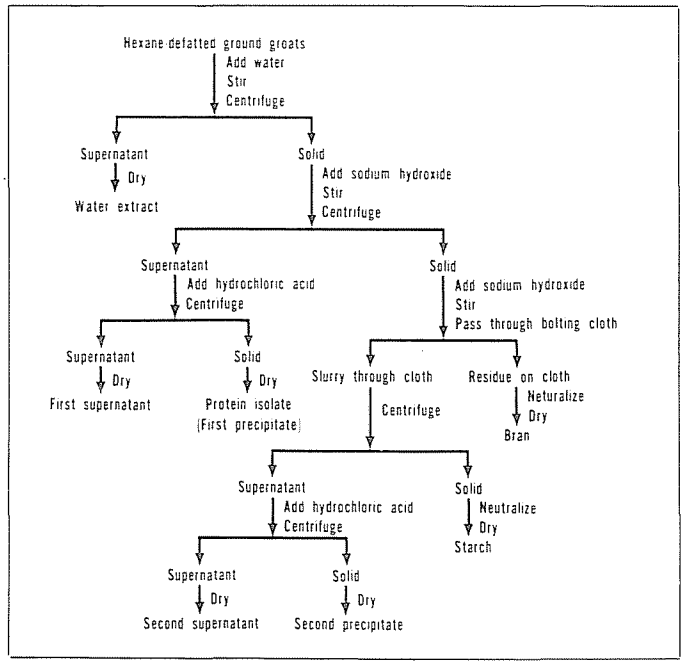

Fig. 2—Schematic diagram for preparing protein isolate and by-products from hexane-defatted ground groats by alkaline extraction.

Composition

Ash and fiber were determined according to AACC Approved Methods (1971), and starch was determined by a polarimetric method (Garcia and Wolf, 1972). Fat was determined by petroleum ether extraction.

Each sample for amino acid analysis was hydrolyzed for 24 hr by refluxing in constant-boiling hydrochloric acid, evaporated to dryness and dissolved in pH 2.2 citrate buffer. A portion of the acid hydrolyzate was put into a Beckman Spinco Model 121 amino acid analyzer, and data were computed automatically (Cavins and Friedman, 1968).

Properties

Nitrogen solubility of isolate was determined by stirring 0.1g of solid with 10 ml of water; sodium hydroxide or hydrochloric acid solution was added dropwise to give a range of pH value from 2.1 to 9.8. The slurry was stirred magnetically for 25 min and centrifuged at 1300 × G (or 27,000 × G, if needed) for 20 min to separate solid and supernatant satisfactorily. The supernatant was analyzed for nitrogen and the percentage of soluble nitrogen was calculated at each pH value. Hydration capacity was determined according to AACC Approved Methods (1971). Emulsifying activity and emulsion stability were measured by the method of Yasumatsu et al. (1972) for a simple system, in which only soybean oil and water were added to the protein isolate.

RESULTS & DISCUSSION

Solvent and pH

A number of solvents were used at a solid:solvent ratio of 1:6 to extract protein from defatted Garland flour (Table 1). Only 9% of the nitrogen was extracted by water; dilute hydrochloric acid extracted 39% of the nitrogen. Sodium hydroxide solutions dissolved more protein as pH of the slurry increased from 7.4 to 10.8. The protein content of the extracted solids (freeze-dried supernatant from centrifugation) reached a maximum of 90% when pH of the slurry was 9.1. A good compromise between high percentage of nitrogen extracted and high protein content of the extracted solid seemed to be around pH 9.1 in 0.02N sodium hydroxide, and these conditions were used subsequently for making the protein isolate.

Products from flour

The yield and composition of protein isolate and by-products from hexane-defatted and butanol-defatted Garland flour are listed in Table 2. The butanol-defatted flour gave a slightly higher yield of protein isolate (12 vs 10%) as well as a little higher protein content (103 vs 101%) compared with hexane-defatted flour. The values of over 100% in the protein content resulted from a conventional conversion factor of 6.25 from nitrogen to protein. Ash and a small amount of fat were also present for oat isolate from butanol-defatted flour.

Table 2—Products from hexane-defatted (H) and from butanol-defatted (B) Garland flour (% dry basis)

	Weight		Protein (N X 6.25)		Total N		Fat		Ash	
Product	H	B	H	B	H	B	H	B	H	B
Protein isolate	10	12	101	103	53	67	[a]	0.2	[a]	1.5
Supernatant	3	4	27.6	31.0	5	7	0.1	0.3	12.1	10.7
Bran	25	2	17.2	14.2	23	2	0.1	0.4	1.6	2.2
Layer above starch	7	18	5.6	9.1	2	9	0.2	0.2	2.5	1.5
Starch	49	56	0.6	0.7	1	2	0.2	0.1	0.5	0.6
Total	94	92			84	87				
Defatted flour			19.2	18.6			0.2	0.2	1.4	1.3

[a] Not determined

Table 3—Products from hexane-defatted Dal groats (% dry basis)

Product	Wt	Protein (N X 6.25)	Total N	Fat	Ash	Starch	Fiber
Water extract	7	27.6	8	0.5	9.9	0	0
Protein isolate (First ppt)	14	93.6	55	1.7	4.0	0	0.1
First supernatant	2	31.6	2	0.3	28.7		
Second ppt	2	90.7	8	2.0	4.8	1.2	0.1
Second supernatant	1	27.1	1	0.4	35.9		
Bran	12	19.5	10	1.1	6.2	8.3	18.5
Starch	51	3.4	7	0.2	5.7	96.6	0.3
Total	89		91				
Defatted groats		24.0		1.5	2.7		2.1

The supernatants contained albumin, globulin, salt, gum and other water-soluble materials, and had about 30% protein, low fat, and relatively high ash. The ash value included the natural salts present in the flour as well as sodium chloride resulting from neutralization of sodium hydroxide and hydrochloric acid solutions. The yield of bran was much higher for hexane-defatted flour than for butanol-defatted flour. The protein, fat and ash contents of bran did not differ greatly from those of the defatted flour. The yield of the layer above starch was considerably lower for hexane-defatted flour than for butanol-defatted flour. The layer above starch had a much lower protein content but similar ash and fat contents compared with the defatted flour. The starch fraction was low in protein, fat and ash. The butanol-defatted flour gave better results than hexane-defatted flour when the yield and protein content of the isolate, as well as the by-products, were considered.

Products from groats

The yield and proximate composition of protein isolate and by-products from hexane-defatted Dal groats are shown in Table 3 and are expressed in percent dry basis. The water extract, first supernatant and second supernatant contained albumin, globulin, gum, salt and other water-soluble materials. These fractions had approximately 30% protein and low fat. The high ash content of the first and second supernatants was due primarily to the sodium chloride resulting from neutralization of sodium hydroxide and hydrochloric acid solutions.

The yield of protein isolate was 14%. This isolate had 94% protein and accounted for 55% of the total nitrogen of defatted groats. The isolate had no starch, low fiber and some fat and ash. When hexane-defatted Dal groats were processed in the laboratory by the same procedure, the protein content of the isolate was 100% (not shown in table). Apparently the laboratory centrifuge was more efficient than the large Sharples centrifuge. The second precipitate was quite similar in composition to the protein isolate.

The bran fraction had high fiber content and contained most of the fiber from the defatted groats. It had lower protein and fat contents but more ash than the defatted groats. The starch fraction had high starch content and low fat and fiber. A layer above starch was not separated from the starch fraction as in Figure 1, otherwise the starch fraction here would have lower protein and ash than the values shown in Table 3.

Amino acid composition

The essential amino acid composition of protein isolates and starting materials were compared with the suggested human requirement (FAO/WHO, 1973) in Table 4. The lysine contents of the flour, groats and isolates were lower than the FAO pattern although higher than most cereals. A preliminary

Table 4—Essential amino acid composition of oat protein isolate and fraction (g/16g nitrogen recovered)

	Defatted Garland flour[a]			(H) Dal groats[a]		FAO/WHO
Amino acid	Flour	Protein isolate (H)	Protein isolate (B)	Groats	Protein isolate	(1973)
Isoleucine	4.2	4.5	4.7	4.0	4.5	4.0
Leucine	7.7	8.3	8.7	7.7	8.3	7.0
Lysine	3.9	4.0	3.5	4.3	3.4	5.5
Methionine + cystine	3.3	2.2	4.2	3.7	2.5	3.5
Phenylalanine + tyrosine	9.6	10.0	11.1	9.8	10.3	6.0
Threonine	3.4	3.5	3.7	3.4	3.3	4.0
Valine	5.7	5.8	6.8	5.7	5.9	5.0

[a] H - hexane-defatted; B - butanol-defatted

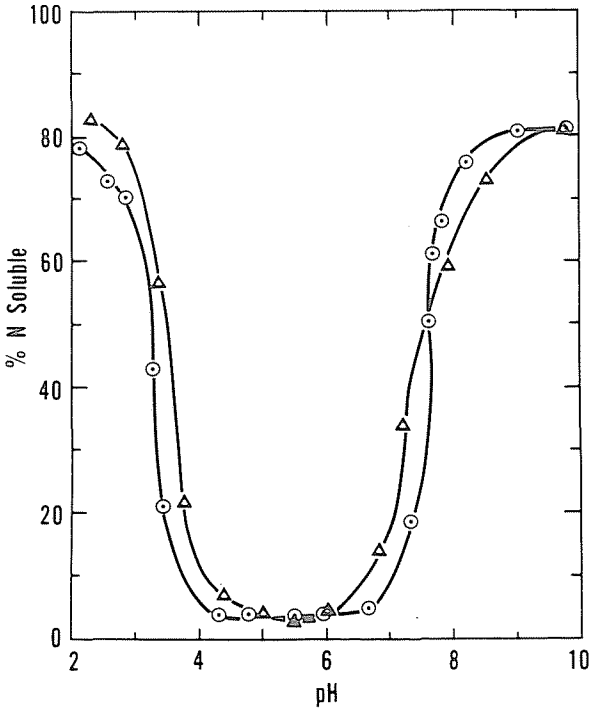

Fig. 3—Nitrogen solubility curve of oat protein isolate from hexane-defatted Dal groats ○ and butanol-defatted Garland flour △.

Table 5—Some functional properties of oat isolate compared with a soy isolate

Protein isolate	Hydration capacity	Emulsifying activity (%)	Emulsion stability (%)
Garland (H)	3.9	28	8
Garland (B)	3.8	51	47
Dal	2.9	47	48
Soy		47	64

water extraction also lowered the lysine content of the isolate from hexane-defatted Dal groats (4.3 to 3.4). However, the isolate from hexane-defatted Garland flour without the water extraction had the same lysine value as the flour.

Although methionine + cystine content of the flour and groats meets the FAO/WHO pattern, the protein isolates from hexane-defatted flour and groats had lower methionine + cystine content. However, the protein isolate from butanol-defatted Garland flour had more than the FAO/WHO pattern for total sulfur amino acids. Threonine levels of flour, groats and protein isolates were all a little lower than the human requirements. The rest of the essential amino acids of flour, groats and isolates meet or exceed the FAO/WHO pattern.

Nitrogen solubility

The minimum nitrogen solubility of Garland isolate was 3% at pH 5.5 (Fig. 3) and the solubility increased rapidly above pH 7 to 81% at pH 9.8. The solubility of Garland isolate also increased rapidly below pH 4 and reached 83% at pH 2.3. Dal isolate had a broader minimum solubility region and lower solubility below pH 4.8 and between pH 6.2 and 7.6 compared with that of Garland isolate. However, the Dal isolate was more soluble than the Garland isolate between pH 7.6 and 9.6. No large difference in nitrogen solubility was observed between isolates made from hexane-defatted Dal groats and from butanol-defatted Garland flour. The difference in nitrogen solubility between Garland isolates made from butanol-defatted and hexane-defatted flours is likely smaller than that shown in Figure 3.

Hydration capacity

Hydration capacity (weight of sediment per weight of sample) of Garland isolate from hexane-defatted and butanol-defatted flour was 3.8 and 3.9, respectively (Table 5). Dal isolate from hexane-defatted groats had a hydration capacity of 2.9. All three isolates had good hydration capacity although Garland isolates gave higher values.

Emulsifying activity and emulsion stability

Garland isolate from hexane-defatted flour had an emulsifying activity of 28% and poor emulsion stability (Table 5). Garland isolate from butanol-defatted flour and Dal isolate from hexane-defatted groats, however, had good emulsifying activity and good emulsion stability. For comparison, the emulsifying activity and emulsion stability of soy isolate were also determined by the same experimental procedure. The emulsifying activity of the soy isolate was about equal to that of the two better oat isolates, and its emulsion stability was better than those of the oat isolates.

Effect of defatting solvents on protein isolates from Garland flour

Butanol removes bound as well as free lipids while hexane removes only free lipids. The removal of bound lipids by butanol resulted in a better separation between protein and starch as evidenced by much lower bran and higher layer above starch weights as well as slightly higher yield of protein isolate and higher protein content of isolate (Table 2). The isolate from butanol-defatted flour also had higher methionine + cystine content (Table 4) and better emulsifying activity and emulsion stability (Table 5).

Potential uses of protein isolate and by-products

Oat protein isolate may find application in foods as a protein ingredient. Some of the isolates had attractive water-binding capacity, good emulsifying activity and good emulsion stability, and these properties suggest possible use as fat emulsifiers and water-absorbing agents in prepared foods. The residue after one protein extraction was successfully extruded in our laboratory and can be used as breakfast cereal or snack food. Alternatively, the residue after one protein extraction may be used as a starch source for fermentation. Pure starch can also be produced (Fig. 1 and 2).

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